Plant Molecular Biology **33:** 71–77, 1997.

Expression characteristics of *OS-ACS1* and *OS-ACS2*, two members of the 1-aminocyclopropane-1-carboxylate synthase gene family in rice (*Oryza sativa* L. cv. Habiganj Aman II) during partial submergence

Thomas I. Zarembinski[1] and Athanasios Theologis*
*Plant Gene Expression Center, 800 Buchanan St., Albany, CA 94710, USA (*author for correspondence);*
[1]*Present address: LBL Calvin Laboratory, University of California, Berkeley, CA 94720, USA*

Received 18 March 1996; accepted in revised form 23 September 1996

Key words: abscisic acid, 1-aminocyclopropane-1-carboxylate synthase, deepwater rice, gibberellic acid, intercalary meristem, submergence

Abstract

Deepwater rice can grow in the regions of Southeast Asia that are flooded during the monsoon season because it has several adaptations allowing it to survive under flooded conditions. One such adaptation is the ability for rapid internode elongation upon partial submergence to maintain its foliage above the rising flood water levels. Ethylene is considered to be the trigger of this growth response because deepwater conditions not only trap ethylene in submerged organs, but also enhance the activity of 1-aminocyclopropane-1-carboxylate (ACC) synthase. Herein we have studied the expression characteristics of two members of the five-member multigene family encoding ACC synthase in rice *OS-ACS1* and *OS-ACS2* and show that partial submergence induces expression of *OS-ACS1* and suppresses expression of *OS-ACS2*. The induction of *OS-ACS1* occurs within 12 h of partial submergence and at low oxygen concentrations. The data also suggest that deepwater conditions posttranscriptionally regulate ACC synthase activity. *OS-ACS1* gene expression may contribute to longer-term ethylene production, but not to the initial, growth-promoting increase in ethylene synthesis.

Abbreviations: IM, intercalary meristem; GA, gibberellic acid; ABA, abscisic acid; ACC, 1-aminocyclopropane-1-carboxylic acid

Introduction

Deepwater rice is cultivated in Southeast Asia because it has the ability to withstand the frequent floodings occurring in this region [18]. When rice plants are partially submerged, they rapidly elongate their younger internodes to keep their foliage above water [18]. The elongation response is quite pronounced; growth rates of up to 25 cm/day and total internodal lengths of 7 m have been recorded [18].

The elongating internode is composed of three contiguous zones, each of which is affected by partial submergence. The intercalary meristem (IM) is located slightly above the second highest or youngest node and is primarily responsible for cell division. The adjacent acropetal zones are the cell elongation and differentiation zones, respectively [3] (see Fig. 1). Partial submergence has three distinct effects on these zones: it increases the IM cell cycle rate three-fold; it causes the IM and the cell elongation zones to expand in length and it suppresses differentiation in the cell differentiation zone [3].

Ethylene is believed to trigger the signal transduction pathway(s) responsible for internode elongation in deepwater rice [18, 22, 24] by altering the responsiveness to and the levels of gibberellic acid (GA) [8, 23, 27] and abscisic acid (ABA) [8]. Partial submergence induces the activity of 1-aminocyclopropane-1-carboxylate (ACC) synthase eight-fold in the 1 cm zone containing the IM (zone 3, Fig. 1) [5], resulting in an increase in ethylene production. Ethylene concentration increases due to both enhanced production

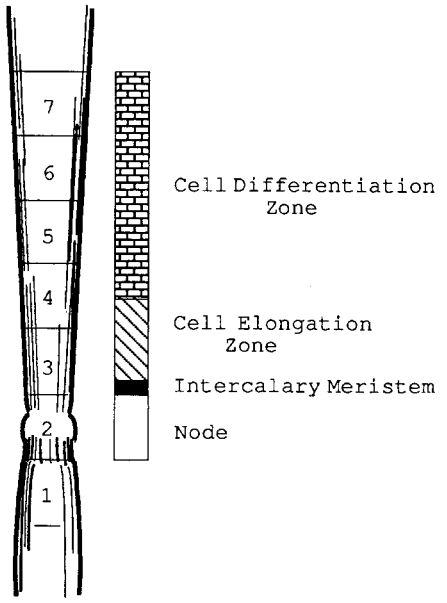

Figure 1. The internode of elongating deepwater rice (from [3] and [5]).

and to the low rate of ethylene diffusion in water [15, 20]. The high ethylene concentration then increases the GA responsiveness [23] and the level of GA [8] while the ABA content decreases in zone 3 [8]; Since the ABA effects are antagonistic to those of GA [8], the effective GA concentration is enhanced more than four-fold. This increase in GA level coupled with the increase in GA responsiveness is believed to induce cell elongation followed by cell division in the IM [27]. Cell elongation in the elongation zone but not in the IM is thought to be due to GA-mediated inhibition of cellulose microfibril reorientation in the internode epidermis [28].

To understand the molecular basis of hypoxia-induced ethylene production, the expression pattern of two members, *OS-ACS1* and *OS-ACS2*, of the at least five-member family encoding ACC synthase under submergence has been investigated [34]. Here we found that only one ACC synthase gene, *OS-ACS1*, is induced in zone 3 after partial submergence. The kinetics of *OS-ACS1* mRNA accumulation during partial submergence suggests that *OS-ACS1*-mediated ethylene production may be important for long-term and sustained ethylene production and growth. Taken together, the data suggests that *OS-ACS1* is a potential candidate responsible for, at least in part, sustaining internodal growth, but not for induction of internodal elongation by submergence.

Materials and methods

Plant material and tissue treatment

Rice seed (*O. sativa* L. cv. Habiganj Aman II) was obtained from the International Rice Research Institute (Manila, Philippines) and was planted in six-inch pots containing prewetted Supersoil (Rod McLellan Co., San Francisco, CA). Four seeds per pot were planted. The pots were placed in waterproof flats in a growth room under thirty-minute interval misting for two weeks. The growth room conditions were the following: day temperature, 26 °C; night temperature, 23 °C; RH, 60%; light photoperiod, 14 h day/10 h night; light intensity, 250 μE m^{-2} s^{-1}. After the two-week period, the plantlets were moved to the non-misted portion of the room. The flats were then watered once per week with 0.5× Hoaglands solution. The flats were also watered daily with tap water so that 8–10 cm of water remained in it. During the winter months (December to February), the feeding of the plants was supplemented three times with a Sequestrene 330 Fe solution (CIBA-Ceigy; 0.15 g/l H_2O) to prevent chlorosis. The first two supplements were given two weeks apart followed by a final supplement one month later.

Two- to three-month-old plants were 70% submerged in large 200 l garbage cans (Rubbermaid) in the same room where they were grown. The tap water used for the submergence studies was warmed to 25 °C. Seven 1-cm regions of the elongating internodes corresponding to zones 1 through 7 were harvested (see Fig. 1) and frozen immediately in liquid N_2 as described [5]. For gassing experiments, leaf sheaths were peeled off to expose the elongating internode. The elongating internode was harvested as described [20]. The sections were placed in foil-covered bell jars and treated with water-saturated gases (AirCo). The different oxygen concentrations were prepared in nitrogen except for the 21% oxygen, which was dry air (AirCo). The 1-cm sections were harvested and frozen immediately in liquid N_2 as described above.

PCR

The sequence and orientation of the gene-specific primers for amplifying the α-amylase cDNA fragment *AMY1* (*AMY1* corresponds to a fragment of the pOS103 cDNA [10]), and the *OS-ACS1-5* are as follows (the numbering of primers is from its genomic sequence

where given):

AMY1-F	5′-GCC GAA TTC GGC TAC ATG CCT GGG GGG C-3′	(2807–2825)
AMY1-R	5′-GCC AAG CTT TGG GTG AGG ATG TAT GCG TA-3′	(3600–3581)
OS-ACS1-F	5′GCC GAA TTC GAT GGT GAG CCA AGT GGT CG-3′	(167–186)
OS-ACS1-R	5′-GAG CGC GTG GGG GTT CTT CT-3′	(490–471)
OS-ACS2-F	5′-GCC CTG CAG AAT GGC CGT GCA GGG CAT CG-3′	
OS-ACS2-R	5′-TTG GCG ATC CTC TTG AAC TG-3′	
OS-ACS3-F	5′-GCC ATC CAG ATG GGC CTT GCA GA-3′	
OS-ACS3-R	5′-CGT CTC CAT GAA GCT CGC CA-3′	
OS-ACS4-F	5′-GCC GTC TCC ATC GAC CTC CTC GA-3′	
OS-ACS4-R	5′-GCC GCC CAG CGC ATT GTC CCT GAA GCT G-3′	
OS-ACS5-F	5′-GCC GTC TCC TTC GAC CTC CTC GAG GC-3′	
OS-ACS5-R	5′-GCC GCC CAG CGC GTT GTC CCT GAA GCT A-3′	

The conditions of the PCR reactions using the TZ-1F and TZ-2R and the gene-specific primers are as previously described [34]; annealing temperature of 55–60 °C was used with the gene-specific primers. The coding region of the *OS-ACS2* cDNA was synthesized using gene specific primers and a first-strand cDNA library from cycloheximide-induced rice shoot tissue. The PCR conditions were identical to those previously used for the synthesis of prACS1 [34].

DNA sequencing

All DNAs were sequenced as previously described [34]. 7-deaza-2′-dGTP was included in all sequencing reactions to resolve compressions. An extension temperature of 42 °C was used to prevent pauses and stops [34].

RNA hybridization analysis

Poly(A)$^+$ RNA was isolated as previously described [34]. The RNAs were glyoxylated, electrophoretically separated, blotted onto Magna nylon membrane (Micron Separations, Westboro, MA), and hybridized with ^{32}P-labeled probes according to manufacturer's instructions. The *17S rRNA* probe was isolated as previously described [34].

Results

Expression of the OS-ACS *genes during partial submergence*

We used a PCR-based approach to determine which *OS-ACS* genes are expressed in elongating deepwater rice internodes after partial submergence. First-strand cDNA libraries were constructed with mRNAs from zones 2 through 6 of 24 h partially submerged, elongating internodes (see Figs. 1 and 2). Subsequently each *OS-ACS* gene was detected using gene-specific primers and PCR conditions described in Materials and Methods. Figure 2 shows that *OS-ACS1* is highly expressed in all zones whereas *OS-ACS2* is weakly expressed only in zones 4–6 (Fig. 2). *OS-ACS4* is expressed at low levels in zones 2–5 (Fig. 2). In contrast, *OS-ACS3* and *5* are not expressed in any of the zones (Fig. 2).

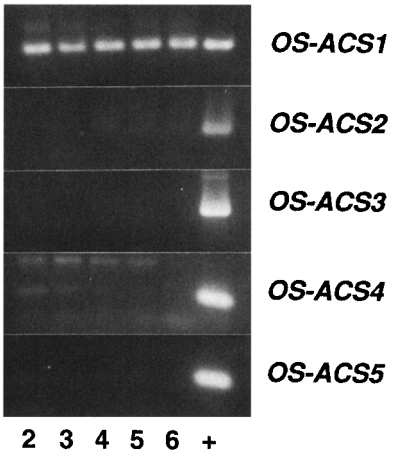

Figure 2. Expression of the rice ACS genes in the various zones of the elongating internode. The figure shows ethidium bromide stained gels of the PCR amplification products using first strand cDNA libraries from elongating deepwater rice internodes. The amplification products were generated from zones 2 through 6 after 24 h of partial submergence. The zone numbers are shown at the bottom.

Induction kinetics under submergence

To obtain more quantitative data on the induction of *OS-ACS*, RNA hybridization analysis was carried out. We have analyzed only the expression of *OS-ACS1* and *OS-ACS2* because of the availability of full-length cDNAs for these genes. Unfortunately we were unable to study the expression of *OS-ACS3–5* because only small-sized genomic fragment were available (0.2 kb), unsuitable for such analysis. The data show that *OS-ACS1* mRNA is induced between 6 and 12 h of partial submergence whereas the *OS-ACS2* mRNA levels are repressed after 24 h of partial submergence (Fig. 3: compare air and submergence). The induction of the rice *ADH1* mRNA level is faster than that of *OS-ACS1*, increasing within 6 h of partial submergence (Fig. 3). The *OS-ACS1* mRNA is also slightly induced in the presence of air (Fig. 3).

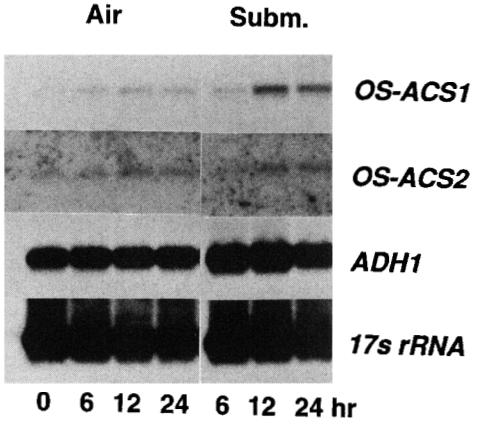

Figure 3. Induction kinetics of *OS-ACS1* and *OS-ACS2* in zone 3 of the submerged internode. The panels show autoradiograms of RNA filters hybridized successively with the following ^{32}P-labelled probes for (A) *OS-ACS1*, (B) *OS-ACS2*, (C), *RAB16A* [19], (D), *AMY1* [10] (E) *ADH1* [33], (F) 17s rRNA [31]. Hybridizations were carried out with 2×10^6 cpm/ml hybridization mixture. Lanes contain 4 μg poly(A)$^+$ RNA.

Zone-specific expression patterns of OS-ACS1 *and* OS-ACS2

RNA hybridization analysis was also carried out to more precisely quantitate the *OS-ACS1* and *OS-ACS2* mRNA levels in each zone after 24 h of partial submergence. The results are shown in Fig. 4. The *OS-ACS1* mRNA is induced five-fold in zone 3 and is repressed in all other zones (Fig. 4 compare zone 3 in air and submergence). In contrast, the *OS-ACS2* mRNA is repressed in all zones except for zone 4 where mRNA levels do not change (Fig. 4). The rice *ADH1* mRNA is strongly induced in all zones after partial submergence, indicating all zones are competent to respond to hypoxia (Fig. 4). Interestingly, the *ADH1* mRNA is highly induced in zone 3 of air-treated control plants (Fig. 4; see discussion).

Effect of oxygen concentration on OS-ACS1 *and* OS-ACS2 *gene expression*

To determine which oxygen concentration induces *OS-ACS* gene expression, deepwater rice stem sections were treated with different concentrations of oxygen (in N_2) for 24 h and northern analysis was done for zone 3. Oxygen concentrations less than 15% induce the *OS-ACS1* mRNA; the strongest induction occurs in the absence of oxygen (Fig. 5). All oxygen concentrations lower than 15% oxygen repress the *OS-ACS2* mRNA with the most dramatic repression in the absence of oxygen (Fig. 5). In contrast, the *ADH1* mRNA is induced at concentrations less than 10% oxygen but is also most strongly induced in the absence of oxygen (Fig. 5). The results of Fig. 5 also show that the expression of *OS-ACS1* is higher in air (21% oxygen) than that at 15% oxygen. This inhibitory effect of lower oxygen concentrations on *OS-ACS1* gene expression is specific because neither *OS-ACS2* nor *ADH1* gene expression is altered under similar treatment (Fig. 5). This may be due to the presence of carbon dioxide (0.03%) in the dry air used for the 21% oxygen treatment; whereas the other oxygen concentrations used were made with pure oxygen in N_2.

GA- and ABA-induced gene expression in zone 3

It has been postulated that submergence-induced ethylene production results in an increase in GA and decrease in ABA concentration [8]. Consequently these hormone changes should lead to alteration of gene expression. Figure 6 shows the effect of partial submergence on the expression of the GA-regulated *AMY1* gene and the ABA regulated *RAB16A* gene. The results show that the *RAB16A* mRNA is moderately repressed under submergence whereas the *AMY1* is repressed. This result indicates the enhanced amylolytic activity in zone 3 after submergence is not linked to the *AMY*1 induction. Since α-amylase is encoded by a large multigene family in rice [10] it is possible that another family member responds to the elevated GA level [8].

Discussion

We have shown that partial submergence induces the *OS-ACS1* mRNA in elongating deepwater rice internodes. *OS-ACS1* mRNA is induced within 12 h of partial submergence in zone 3 and at oxygen concentrations of 2.5% oxygen or less. The 5-fold *OS-ACS1* mRNA induction parallels the ACC accumulation in this zone (data not shown). The *OS-ACS1* mRNA induction at oxygen concentrations less than 15% oxygen suggesting that oxygen tension may modulate a signal transduction mechanism leading to an enhancement in gene expression in submerged deepwater rice internodes. In maize protoplasts, the maize *ADH1* and aldolase promoters are transcriptionally activated at oxygen concentrations of 5% [6, 9]. Whether hypoxia

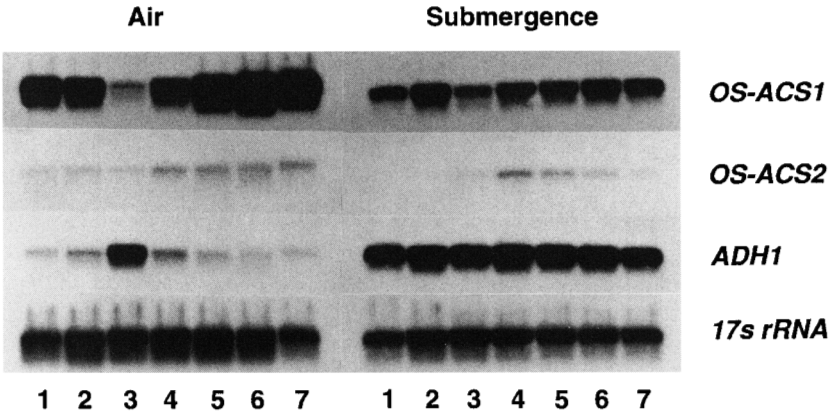

Figure 4. Zone-specific expression of *OS-ACS1* and *OS-ACS2* genes. The panels show autoradiograms of RNA filters successively hybridized with the following ^{32}P-labeled probes for (A) *OS-ACS1*, (B) *OS-ACS2*, (C) *ADH1* [33], and (D) *17s rRNA* [31]. 'Air' and 'submergence' denote the treatment of the various zones (1 cm long) from the elongating internode shown at the bottom of the figure. Hybridizations were carried out with 2×10^6 cpm/ml hybridization mixture. All lanes contain 4 μg poly(A)$^+$ RNA.

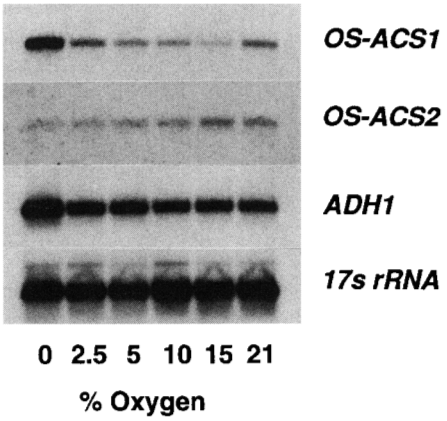

Figure 5. Effect of oxygen concentration of the expression of *OS-ACS1* and *OS-ACS2* in zone 3 of elongating internode. The panels are autoradiograms of RNA filters successively hybridized with the following ^{32}P-labeled probes for (A) *OS-ACS1*, (B) *OS-ACS2*, (C) *ADH1* [33], (D) *17s rRNA* [31]. Each lane contains 4 μg poly(A)$^+$-RNA.

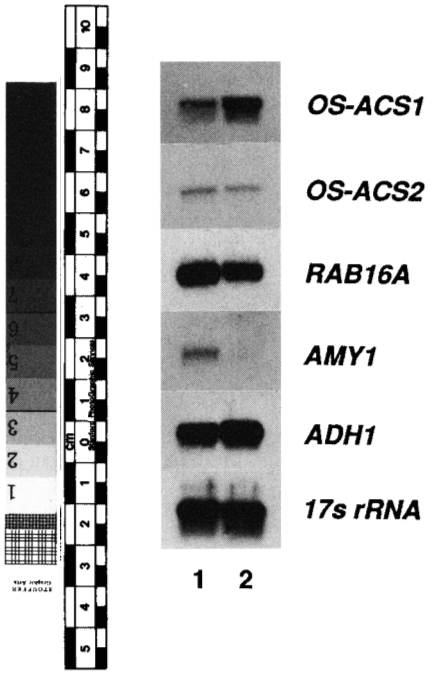

Figure 6. Effect of submergence on the expression of GA- and ABA-regulated genes in zone 3 of the elongating internode. The panels are autoradiograms of RNA filters successively hybridized with the following ^{32}P-labeled probes for (A) *OS-ACS1*, (B) *OS-ACS2*, (C) *RAB16A* [19], (D) *AMY1* [10], (E) *ADH1* [33], (F) *17s rRNA* [31]. The lanes are: 1, air; 2, partial submergence (24 h). All hybridizations were carried out with 2×10^6 cpm per ml hybridization mixture. Each lane contains 5 μg poly (A)$^+$ RNA.

transcriptionally activates the *OS-ACS1* gene remains to be determined.

The constitutive expression of *ADH1* gene in zone 3 of non-submerged internodes (Fig. 3) may indicate hypoxic conditions. Meristems are known to lack oxygen because of low oxygen permeability [20] and high metabolic activity [32]. This result agrees with recent work showing constitutive Maize *ADH1* expression in the shoot and root meristems of transgenic rice plants [13] and high ADH activity in untreated tree vascular cambium [12].

The induction of *OS-ACS1* mRNA is unsheathed elongating stem sections at 2.5% oxygen after 24 h in

the dark does not agree with previous data showing that oxygen levels in intact stems are at their lowest around 4% after 24 partial submergence in a 12 h day-night regimen [30]. This discrepancy may be due to the fact that we were not using intact plants but stem sections with their surrounding leaf sheaths stripped off. Previous studies have shown that growth is inhibited without the sheaths [23]. Another possibility is that other gases within the internal air spaces of the elongating internode may further induce the *OS-ACS1* mRNA. In our experiment (with the exception of the 21% oxygen sample which was gassed with dry air) the stem sections were gassed with only oxygen mixed with nitrogen. Since carbon dioxide and ethylene are known to affect gene expression in plants and other organisms [15, 16, 25], it will be interesting to see whether these two gases which are induced within the intact submerged internode also induce *OS-ACS1* expression.

Our results and those previously obtained by Rose-John and Kende [24] suggest that short-term internodal growth is primarily due to ethylene accumulation from basal production of the hormone while long-term growth may be due to *OS-ACS1* gene expression. Internodal ethylene concentrations are induced within 2 h and internodal growth rate starts to increase after 3 h of partial submergence [24]. Both internodal ethylene production and growth cease after approximately 10 h of submergence and then begin to increase slowly after 24 h [24]. Assuming that no other *OS-ACS* genes are induced by hypoxia within 24 h of partial submergence, the poor diffusion of basal ethylene into the surrounding water most likely causes an accumulation of ethylene around the submerged tissues; this provides the initial ethylene and growth bursts. Only the ethylene increase after 24 hours of partial submergence may be attributed to the *OS-ACS1* mRNA induction since this induction occurs after 6 hr of partial submergence at the earliest (Fig. 5).

Three observations suggest that ethylene production is not only regulated by the availability of the *OS-ACS1* mRNA in submerged deepwater rice. First, prior to submergence, the *OS-ACS1* mRNA is highly expressed in most intenodal zones (Fig. 3), but there is very low ACC production in every zone [5]. Second, while the ACC concentration increases in all zones after submergence [5], the *OS-ACS1* mRNA increases only in zone 3 (Fig. 3). Third, the ACC synthase activity in zone 3 increases earlier (after 4 h of partial submergence) [5], than the observed increase in *OS-ACS1* mRNA (6–12 h; see Fig. 4). These observations suggest the following mechanisms for the regulation of the rice ACC synthase activity: (1) an unidentified hypoxically inducible *OS-ACS* gene (s) is responsible for the large ACC accumulation in zones 4 to 7; (2) hypoxia may regulate other reactions in the ethylene biosynthetic pathway; (3) there may be a post-transcriptional regulation of *OS-ACS1* and *OS-ACS2*; and (4) ACC is synthesized in zone 3 but transported acropetally. As for the first possibility, the cloning of ACC synthase genes by PCR using degenerate primers is biased and therefore not exhaustive [1, 14, 34]. The presence of several new *OS-ACS* cDNAs in the EST database supports this possibility. The second view represents the best explanation for the data since the activity of rice enzyme responsible for the ACC to ethylene conversion, ACC oxidase, is induced by partial submergence [17].

Post-transcriptional regulation of *OS-ACS* activity is also highly possible because there are examples of translational control of hypoxically-inducible proteins and post-translational control of ACC synthase. First, anaerobiosis inhibits translation of aerobic but not of anaerobic mRNAs [7, 26]. It is possible that *OS-ACS* mRNAs are preferentially translated in zones 4 through 7 after partial submergence, resulting in an increase in ACC levels independent of *OS-ACS* mRNA accumulation. Second, a post-translational regulatory mechanism for *OS-ACS* ACC synthase activity may be by phosphorylation of the polypeptide, resulting in inhibition of its turnover [29]. Since both *OS-ACS1* and *OS-ACS2* mRNA are present in zones 4 through 7 (Fig. 3), either or both of their proteins encoded could be phosphorylated during hypoxia, giving rise to high ACC levels. Finally, the ACC induction in the absence of *OS-ACS* mRNA induction in the different zones can be explained by the acropetal transport of ACC; such movement has been shown to occur in waterlogged tomato roots [4]. It is therefore plausible that ACC production is induced in zone 3, but transported to zones 4 through 7.

Conclusion

OS-ACS1 may be one of the rice ACC synthase gene responsible at least in part for long-term partial submergence-induced ethylene production in deepwater rice. This proposition is supported by the specific induction of *OS-ACS1* in zone 3 within 12 hr of partial submergence. Reverse genetic experiments will provide conclusive evidence whether *OS-ACS1* is

indeed the key gene responsible for internodal elongation.

Acknowledgments

We would like to thank Dr Hans Kende for critical evaluation of our manuscript and sending us unpublished data. We also thank Drs Steffen Abel and Klaus Harter for critical reading of the manuscript and Drs Ray Wu (Cornell University), Robert McGrath and Nam-Hai Chua (Rockefeller University) for providing the *ADH1* and *RAB16A* clones, respectively. This work was supported by grants to AT from the National Science Foundation (MCB 9316475) and USDA/ARS (CRIS 5335-21430-003-00D).

References

1. Abel S, Nguyen MD, Theologis A: The *PS-IAA4/5*-like family of early auxin-inducible mRNAs in *Arabidopsis thaliana*. J Mol Biol 251: 533–549 (1995).
2. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ: Basic local alignment search tool. J Mol Biol 215: 403–410 (1990).
3. Bleecker AB, Schuette JL, Kende H: Anatomical analysis of growth and developmental patterns in the internode of deepwater rice. Planta 169: 490–497 (1986).
4. Bradford KJ, Yang SF: Xylem transport of 1-aminocyclopropane-1-carboxylic acid, an ethylene precursor, in waterlogged tomato plants. Plant Physiol 65: 322–326 (1980).
5. Cohen E, Kende H: In vivo 1-aminocyclopropane-1-carboxylate synthase activity in internodes of deepwater rice. Plant Physiol 84: 282–286 (1987).
6. Dennis ES, Gerlach WL, Walker JC, Lavin M, Peacock WJ: Anaerobically regulated aldolase gene of maize: a chimaeric origin? J Mol Biol 202: 759–767 (1988).
7. Freeling M, Bennett DC: Maize Adh1. Annu Rev Genet 19: 297–323 (1985).
8. Hoffmann-Benning S, Kende H: On the role of abscisic acid and gibberellin in the regulation of growth of rice. Plant Physiol 99: 1156–1161 (1992).
9. Howard EA, Walker JC, Dennis ES, Peacock WJ: Regulated expression of an alcohol dehydrogenase 1 chimeric gene introduced into maize protoplasts. Planta 170: 535–540 (1987).
10. Huang N, Sutliff TD, Litts JC, Rodriguez RL: Classification and characterization of the rice α-amylase multigene family. Plant Mol Biol 14: 655–668 (1990).
11. Keith KA, Raskin I, Kende H: A comparison of the submergence response of deepwater and non-deepwater rice. Plant Physiol 80: 479–482 (1986).
12. Kimmerer TW, Stringer MA: Alcohol dehydrogenase and ethanol in the stems of trees. Plant Physiol 87: 693–697 (1988).
13. Kyozuka J, Fujimoto H, Izawa T, Shimamoto K: Anaerobic induction and tisue-specific expression of maize Adh1 promoter in transgenic rice plants and their progeny. Mol Gen Genet 228: 40–48 (1991).
14. Liang X, Abel S, Keller JA, Shen NF, Theologis A: The 1-aminocyclopropane-1-carboxylate synthase gene family of *Arabidopsis thaliana*. Proc Natl Acad Sci USA 89: 11046–11050 (1992).
15. Lincoln JE, Fisher RL: Diverse mechanisms for the regulation of ethylene-inducible gene expression. Mol Gen Genet 212: 71–75 (1988).
16. Ludin KM, Hilti N, Schweingruber ME: *Schizosaccharomyces pombe* rds1, an adenine-repressible gene regulated by glucose, ammonium, phosphate, carbon dioxide and temperature. Mol Gen Genet 248: 439–445 (1995).
17. Mekhedov SL, Kende H: Submergence enhances expression of a gene encoding 1-aminocyclopropane-1-carboxylate oxidase in deepwater rice. Plant Cell Physiol 37: 531–537 (1996).
18. Metraux J-P, Kende H: The role of ethylene in the growth response of submerged deep water rice. Plant Physiol 72: 441–446 (1983).
19. Mundy J, Chua N-H: Abscisic acid and water-stress induce the expression of a novel rice gene. EMBO J 7: 2279–2286 (1988).
20. Perata P, Alpi A: Plant responses to anaerobiosis. Plant Sci 93: 1–17 (1993).
21. Raskin I, Kende H: Effect of submergence on translocation, starch content and amylolytic activity in deep-water rice. Planta 162: 556–559 (1984).
22. Raskin I, Kende H: regulation of growth in stem sections of deep-water rice. Planta 160: 66–72 (1984).
23. Raskin I, Kende H: Role of gibberellin in the growth response of submerged deepwater rice. Plant Physiol 76: 947–950 (1984).
24. Rose-John S, Kende H: Short-term growth response of deepwater rice to submergence and ethylene. Plant Sci 38: 129–134 (1985).
25. Rottmann WH, Peter GF, Oeller PW, Keller JA, Shen NF, Nagy BP, Taylor LP, Campbell AD, Theologis A: 1-Aminocyclopropane-1-carboxylate synthase in tomato is encoded by a multigene family whose transcription is induced during fruit and floral senescence. J Mol Biol 222: 937–962 (1991).
26. Sachs MM, Ho T-H-D: Alteration of gene expression during environmental stress in plants. Annu Rev Plant Physiol 37: 363–376 (1986).
27. Sauter M, Kende H: Gibberellin-induced growth and regulation of the cell division cycle in deepwater rice. Planta 188: 362–368 (1992).
28. Sauter M, Seagull RW, Kende H: Internodal elongation and orientation of cellulose microfibrils and microtubules in deepwater rice. Planta 190: 354–362 (1993).
29. Spanu P, Grosskopf DG, Felix G, Boller T: The apparent turnover of 1-amonicyclopropane-1-carboxylate synthase in tomato cells is regulated by protein phosphorylation and dephophorylation. Plant Physiol 106: 529–535 (1994).
30. Stunzi JT, Kende H: Gas composition in the internal air spaces of deepwater rice in relation to growth induced by submergence. Plant Cell Physiol 30: 49–56 (1989).
31. Takaiwa F, Oono K, Sugiura M: The complete nucleotide sequence of a rice *17s rRNA* gene. Nucl Acids Res 12: 5441–5448 (1984).
32. Vartapetian BB: Introduction-life without oxygen: In: Hook D.D., Crawford RMM (eds) Plant Life in Anaerobic Environments, pp. 1–13. Ann Arbor Science Publishers, Ann Arbor, MI (1978).
33. Xie Y, Wu R: Rice alcohol dehydrogenase genes: anaerobic induction, organ specific expression and characterization of cDNA clones. Plant Mol Biol 13: 53–68 (1989).
34. Zarembinski TI, Theologis A: Anaerobiosis and plant growth hormones induce two genes encoding 1-aminocyclopropane-1-carboxylate synthase in rice (*Oryza sativa L.* Mol Biol Cell 4: 363–373 (1993).